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THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

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Application for Research Grant
(Use extra pages as needed)

Date: 6/30/75

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3. Department(s) where research will be done or collaboration provided:
Division of Microbiology, Department of Laboratories

4. Short title of study: Influence of Cigarette Smoke on the Response of the Alveolar Macrophage System to Inhaled Bacteria.

5. Proposed starting date: January 1, 1976

6. Estimated time to complete: 3 years

7. Brief description of specific research aims: The proposed project emanates from observations made in this and other laboratories which demonstrate the following: (a) during in vivo smoke-exposure or incubation in bronchopulmonary fluid obtained from the lungs of smoke-exposed animals, alveolar macrophages do not effectively kill bacteria and (b) in contrast, alveolar macrophages from long term cigarette smokers and smoke-exposed animals rapidly ingest and destroy bacteria when challenged with bacteria in the absence of cigarette smoke or smoke-products. Although important, these observations fail to provide a scientific basis for explaining the impairment of macrophage function by smoke and the subsequent recovery of alveolar macrophages from the adverse effects of in vivo smoke-exposure. To this end, methods and protocols are presented to distinguish between a direct effect of cigarette smoke on the phagocytic properties of alveolar macrophages and those related to a suppression of phagocytosis-promoting factors present in the lung. To accomplish these objectives, the following determinants of effective macrophage function, as a lung phagocyte, will be studied in the absence of cigarette smoke, during in vitro and in vivo smoke-exposure and after the cessation of smoke inhalation: (a) chemotactic responsiveness to bacterial stimuli, (b) phagocytosis including the adherence of bacteria to macrophage surfaces and the rate at which membrane-associated bacteria are ingested, (c) the rate of intracellular destruction of ingested bacteria and (d) the availability and immunologic competence of phagocytosis-promoting factors present in serum and the lungs.

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8. Brief statement of working hypothesis:

Epidemiologic and clinical reports suggests an increased incidence of respiratory infections among smokers, especially heavy smokers. In support of such a relationship, experimental studies demonstrate that acute and chronic exposure to cigarette smoke impairs the clearance of inhaled bacteria from the lungs of animals. The alveolar macrophage has been implicated as the most important intrapulmonary defense mechanisms which protects the host against bronchopulmonary damage by inhaled infectious and toxic agents. Repeated human and animal studies indicate that cigarette smoke increases rather than depletes the available supply of alveolar macrophages in the lung and does not impair cell viability or the mobilization of macrophages in response to the inhalation of live bacteria. In addition, alveolar macrophages obtained from smokers and smoke-exposed animals retain their ability to effectively kill bacteria, in vitro, in the absence of smoke. In contrast, it has been shown that during in vitro and in vivo exposure to cigarette smoke the ability of alveolar macrophages to destroy bacteria is compromised. These data suggest a direct effect of smoke on the phagocytic and/or bactericidal properties of macrophages. In support of this view, recent studies performed in this laboratory under test conditions that dissociate phagocytosis from bacterial destruction demonstrate that in vitro smoke-exposure has an adverse effect on the rate at which bacteria bound to macrophage membranes are ingested and destroyed. These findings must be confirmed under in vivo conditions of smoke-exposure in the absence of possible artifacts. For this reason a significant part of the proposed research is devoted to an evaluation of the effects of in vivo smoke-exposure on phagocytic and bactericidal activity studied as sequential and independent processes. The data obtained from other studies also suggest that impairment of macrophage function by smoke inhalation may be mediated by a smoke-occasioned augmentation of the supply and/or immunologic integrity of phagocytosis-promoting factors present in the lung. In support of this view are two fundamental observations made in this laboratory: (1) in the absence of normal serum or immunologically active components of the lung obtained by bronchopulmonary lavage, alveolar macrophages do not kill bacteria, in vitro, and (2) bacterial challenge in bronchopulmonary lavage material obtained from smoke-exposed animals results in a marked suppression of the antibacterial properties of alveolar macrophages. These findings indicate the need for a direct investigation of the immunological accompaniments that add to the functional activity of alveolar macrophages in the absence of smoke, during smoke-exposure and after the cessation of smoke inhalation. Finally, the clinical significance of the data obtained from the proposed investigation is strengthened by the fact that the alveolar macrophage and immune systems in the respiratory tract of animals appear to respond to cigarette smoke in a manner similar to that of man.

See pages 16 to 36 for a summary of the Background Material and Supporting Data that serve as a basis for the studies proposed in this grant.

9. Details of experimental design and procedures (append extra pages as necessary). See pages 2A-1 to 2A-13.

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9. Experimental Design: Study of the Influence of Cigarette Smoke on the Phagocytic Properties of Alveolar Macrophages.

Reference Response System: Effective Phagocytosis and Destruction of *Staphylococcus aureus* in an *In Vitro* Phagocytosis System

Conditions of Smoke Exposure:

- *In Vitro*: Normal Alveolar Macrophages Exposed to Cigarette Smoke in Tissue Culture Flasks
- *In Vivo*: Alveolar Macrophages Harvested from the Lungs of Rabbits Exposed to Cigarette Smoke

Test Conditions of Bacterial Challenge:

- Tissue Culture Media plus Normal and Immune Rabbit Serum
- Tissue Culture Media plus the Immunologic Components in the Lung Retrieved from the Lungs of Normal and Immune Rabbits by Bronchopulmonary Lavage

Parameters of Phagocytosis to be studied:

1. Chemotactic Responsiveness to Bacterial Stimuli
2. Attachment of Bacteria to Macrophage Surfaces
3. Ingestion of Membrane Associated Bacteria
4. Intracellular Destruction of Ingested Bacteria by Macrophages

The Concentration and Activity of Mediators of Phagocytosis in Serum and Lung to be Assayed:

Serum	Lung
1. Complement (C'3, C'5)	1. Complement (C'3, C'5)
2. IgG	2. IgG
	3. SIgA

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Experimental Design

A. Quantitative Measurements of the Influence of Cigarette Smoke on Alveolar Macrophage Activity: There is sufficient evidence to suggest that the alveolar macrophage is critically important in pulmonary defense against inhaled bacteria. The specific components of alveolar macrophage activity, as a lung phagocyte, which enable it to serve this key role in pulmonary defense have been demonstrated. Alveolar macrophages are mobilized following the inhalation of bacteria and can effectively ingest and destroy bacteria deposited in the lungs. Accordingly, studies are presented to assess the effect of cigarette smoke on the chemotactic and phagocytic properties of alveolar macrophages. The proposed studies were designed to assess the individual and comparative effects of in vivo and in vitro smoke-exposure on the antibacterial activity of alveolar macrophages, and the phagocytosis-promoting properties of serum and the immunologic components of the lung retrievable by bronchopulmonary lavage. To accomplish these objectives, all studies will be performed both under in vitro and in vivo conditions of smoke-exposure and will include alveolar macrophages from non-immunized rabbits challenged with Staphylococcus aureus in tissue culture media individually supplemented with one of the following additions: (a) normal rabbit serum, (b) serum from rabbits immunized against S. aureus, (c) bronchopulmonary lavage from normal rabbits and (d) bronchopulmonary lavage from rabbits immunized against S. aureus. This basic approach is consistent with the specific aim of the grant proposal to distinguish between smoke-associated changes in the antibacterial activity of alveolar macrophages due to a direct effect of cigarette smoke on the alveolar macrophage proper and those ascribable to a smoke-induced augmentation of the immunological accompaniments necessary for effective antibacterial action by alveolar macrophages. This information is also needed to provide a scientific basis for explaining the impairment of macrophage function by cigarette smoke and the rapid recovery of alveolar macrophages from the adverse effects of smoke inhalation.

The conditions of in vivo and in vitro exposure to cigarette smoke, in vitro bacterial challenge and methods for harvesting alveolar macrophages, collecting the immunologic components of the lung by bronchopulmonary lavage and immunization schedules are presented in detail under Methods of Procedure on pages 2A-8 to 2A-11.

(1) Studies of the Chemotactic Responsiveness of Alveolar Macrophages: An understanding of the influence of cigarette smoke on chemotactic responsiveness is desirable because the rapid mobilization and migration of alveolar macrophages to pulmonary sites following the inhalation of infectious and toxic agents determines the prevention and limitation of bronchopulmonary damage from these materials. The data obtained from these studies will be correlated with observation made in this laboratory (1,2) which demonstrate that cigarette smoke induced a selective mobilization of alveolar macrophages and did not interfere with the mobilization of alveolar macrophages provoked by the inhalation of viable S. aureus

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Since the chemotactic response to a given stimulus is the adjusted composite of changes in cell adhesiveness and motility, both properties of the alveolar macrophage will be investigated. The experimental system used by Carruthers (3) to study leucocyte motility will be adapted to alveolar macrophages. This method is based on the ability of motile cells to move through the pores of a membrane filter. Two O-ring joints separated by a millipore filter are clamped together and sealed to form 2 distinct chambers. Fixed numbers of alveolar macrophages ($1-3 \times 10^7$) suspended in tissue culture media will be introduced into chamber number one which is then sealed with a paraffin plug. The second chamber will be filled with 5 mg. of insoluble potato starch. After an initial period of incubation at 37°C to permit monolayer formation, the chambers will be inverted so that the test cells will then be on the bottom side of the filter, and the chemotactic substance, if present, is on the top side of the filter. The chamber will then be placed in an incubator at 37°C for 4 hours. At the end of various hourly intervals, the filter will be removed, stained with hematoxylin and subjected to microscopic study. In this way, the number of cells on both sides of the filter will be enumerated and used as an index of the migratory response elicited by stimulatory agents. Separate studies are planned to evaluate the changes in cell adhesiveness that occur in phagocytizing alveolar macrophages. The method of studying the adhesive properties of blood leukocytes reported by Allison and Lancaster (4) will be adapted to alveolar macrophages. Test tube cultures of fixed numbers of lung phagocytes suspended in tissue culture media will be challenged with known numbers of S. aureus. Under conditions favoring maximum phagocytosis, microscopic methods will be used to determine the formation of cell aggregates by phagocytizing macrophages. Changes in cell adhesivenss will be evaluated under the same experimental conditions described above to assess motility.

(2) Studies of Phagocytosis and Bacterial Killing: Previous investigations have established the importance of phagocytosis and bacterial destruction by alveolar macrophages in the initial defense of the lung against bacterial invasion. Phagocytosis and bacterial killing are sequential events that must be measured simultaneously to accurately determine the overall antibacterial properties of a phagocytic cell. At present, it is also possible to dissociate phagocytosis from bacterial destruction so that each process can be investigated independently. Failure to utilize methods that permit quantitative determinations of overall antibacterial activity, phagocytosis and intracellular inactivation of phagocytized bacteria can and have lead to misleading reports.

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(a) Simultaneous Measurements of Phagocytosis and Bacterial Destruction: Fixed numbers of S. aureus are added to known numbers

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of alveolar macrophages adhering to the flat surface of a tissue culture flask containing tissue culture media. Immediately after bacterial challenge and at various intervals thereafter, the extracellular fraction containing free or unphagocytized bacteria is isolated by removing the supernatant fluid from macrophage cultures with a pipette. To recover the viable bacteria within macrophages (macrophage fraction), the tissue culture flasks are vigorously shaken after adding glass beads and the bacteria-laden macrophages are lysed by treatment with cold distilled water. In all studies the protocol included control flasks containing the tissue culture medium inoculated with S. aureus. Bacterial counts are obtained from the extracellular fraction, macrophage fraction and control flasks by a standard pour plate technique. The rate of disappearance of viable organisms from the intracellular fraction is used to express the percent bacteria phagocytized, while the difference between the total viable bacterial population in control flasks and the numbers of bacteria in the extracellular and macrophage fractions is used to determine the percent bacteria killed by alveolar macrophages.

(b) Phagocytosis: Since phagocytosis has been shown to be a two stage event consisting of the adherence to and subsequent destruction of ingested bacteria by phagocytes, methods will also be used to differentiate bacterial attachment from ingestion. After challenging macrophages cultures with S. aureus for 1.5 hrs, the macrophage fraction is separated from the extracellular fraction, as previously described, and incubated for 15 min in 3.0 ml of tissue culture medium containing 2.5% trypsin (Grand Island Biological Company). The bacteria released from the surface of macrophages by trypsin (adherence fraction) are recovered by decanting the supernatant and washing the macrophages three times with 2.5 ml of Hanks' solution. The bacteria associated with the trypsin-treated macrophages are present within macrophages and may be referred to as the ingested fraction of the total bacteria recovered. To recover the ingested fraction of bacteria, the trypsin-treated macrophages will be lysed by treatment with cold sterile distilled water. The numbers of viable bacteria present in the adherence and ingested fractions are determined from bacterial counts obtained from nutrient agar pour plates incubated at 37°C for 48 hrs. Under these conditions, it is possible to determine the relative number of viable bacteria attached to and within alveolar macrophages (ingested) at various times during the phagocytic event: Ratio of the no. viable bacteria in the adherence fraction to the no. viable bacteria in the ingested fraction.

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(c) Bacterial Inactivation - Intracellular Destruction of Ingested Bacteria: In other studies, intracellular destruction of ingested S. aureus by alveolar macrophages will be studied in the absence of all other aspects of the phagocytic event. For this purpose

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lysostaphin (Schwartz Mann) will be used (final concentration of 150 ug/ml) to kill all extracellular bacteria as well as those adhering to macrophage membranes. Lysostaphin is a muralytic enzyme which does not enter phagocytes and selectively eliminates extracellular staphylococci (5). Macrophage cultures are challenged with S. aureus for 1 hr at 37°C. At this time, 0.1 ml of lysostaphin is added to all macrophage cultures. After 30 minutes incubation at 37°C, duplicate flasks will be removed from the incubator and treated with 0.3 ml of trypsin to inactivate and neutralize the lysostaphin. Viable intracellular bacteria will be released by agitation with glass beads and osmolysis with cold sterile distilled water. Two hours later, duplicate flasks will be taken out of the incubator as outlined above. Viable bacterial counts will be made 48 hrs later and the % intracellular killing of S. aureus is determined as follows:

$$\text{Intracellular killing} = 100\% - \frac{\text{No. viable bacteria in macrophage cultures 2.0 hrs after bacterial challenge period}}{\text{No. viable bacteria in macrophage cultures immediately after bacterial challenge period}} \times 100$$

THE ABOVE METHODS ARE CURRENTLY IN USE IN THIS LABORATORY

B. Quantitative Measurements of the Influence of Cigarette Smoke on Phagocytosis-Promoting Factors in Serum and Extractable from the Lung by Bronchopulmonary Lavage: There is evidence to suggest that cigarette smoking alters the relative distribution of the immunoglobulins and complement proteins in serum, tracheobronchial mucosa and lung (6,7). The C'3 and C'5 complement proteins and the immunoglobulin IgG and SIgA are of specific interest for the following reasons: (a) C'3 and C'5 are the principle complement components responsible for the chemotactic responsiveness of phagocytes to bacterial agents (8), (b) C'3 and IgG are the best characterized opsonins found in serum and bronchial fluid. In this regard, IgG has been shown to increase the rate at which bacteria attach to receptor sites on the surface of phagocytes, and IgG and C'3 enhance the rate of bacterial ingestion by phagocytes (9) and (c) SIgA is the major secretory immunoglobulin produced locally in tracheobronchial mucosa. Because of its ability to alter bacterial growth rates (10), bacterial viability (11) and the adherence of bacteria to mucosal surfaces (12), SIgA may be a central factor in the role of the alveolar macrophage as a lung phagocyte. For these reasons studies are proposed to assess the effect of cigarette smoke on the concentration and activity of the above humoral factors in serum and bronchial fluid. This information is needed to differentiate between smoke-occasioned changes in macrophage function due to a direct effect of cigarette smoke on the alveolar macrophage and those ascribable to an augmentation of the immunological accompaniments necessary for effective antibacterial action by alveolar macrophages.

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(1) Studies of the Influence of Cigarette Smoke on the Concentration of Immunoglobulins and Complement Proteins Extractable from the Lung by Bronchopulmonary Lavage: The relative distribution of C'3, C'5, IgG and SIgA will be determined in normal rabbits and rabbits immunized against S. aureus prior to and after exposure to cigarette smoke for 1, 5, 10 and 15 days. Quantitation of the humoral factors in question will be made by electroimmunodiffusion as outlined under Materials and Methods of Procedure on page 2A-10.

(2) Studies of the Influence of Cigarette Smoke on the Phagocytosis-Promoting Activity of the Immunoglobulins IgG and SIgA and Complement Components C'3 and C'5: To accomplish the above, experiments will be performed to establish the individual and relative contributions of C'3, C'5, IgG and SIgA to the antibacterial activity of alveolar macrophages. For this purpose, separate studies will be performed to compare the ability of alveolar macrophages to phagocytize and kill S. aureus in serum and bronchopulmonary lavage fluid prior to and after treatment of the serum and bronchial fluid with individual inhibitors and/or absorbants of C'3, C'5, IgG and SIgA. The SIgA determinations apply only to the bronchopulmonary lavage studies. In the course of these studies, direct measurements will also be made of the activity (immunologic competence) of each of the immunoglobulins and complement components by equivalence point titrations against their respective antisera. The selective inhibition and immunoglobulin-complement activity studies will be performed with alveolar macrophages harvested from non-immune animals and challenged with S. aureus under the following conditions of bacterial challenge: (a) in Hanks' solution containing serum from either normal rabbits or rabbits immunized against S. aureus prior to and after in vitro exposure of the sera to various concentrations of whole cigarette smoke and (b) in bronchopulmonary fluid obtained from either normal rabbits or rabbits immunized against S. aureus not exposed to cigarette smoke, immediately after in vivo exposure to cigarette smoke and after the termination of experimental smoke inhalation.

The methods used to achieve selective depletion and/or absorption of each humoral component and technique used to perform equivalence point titrations are presented in detail on pages 2A-11.

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Materials and Methods of Procedure

A. Conditions of In Vivo Smoke-Exposure: A continuous stream of puffed cigarette smoke generated from commercial non-filtered 70 mm cigarettes is produced by an automatic smoke machine* designed to sequentially puff 30 cigarettes to a 20 to 23 mm butt length. The smoke generating apparatus is adjusted to deliver a 35 ml puff of 2 sec duration from each cigarette once every min. Cigarette smoke is drawn through a smoke-exposure chamber at a rate of 25 liter per min by a secondary airflow of room air created by a vacuum pump located at the downstream end of the exposure chamber. Airflow is monitored with a rotometer and controlled to produce chamber concentrations of cigarette smoke of approximately 1 part of whole cigarette smoke and 25 to 30 parts room air. Control animals are placed in a chamber and sham-smoked with unlighted cigarettes and subjected to a secondary airflow of room air.

In the present studies Albino New Zealand male rabbits will be exposed daily for 1.0 hr to whole cigarette smoke for 1, 5, 10 and 15 days over a 3 week period. These are the same conditions of smoke-exposure used in this laboratory to assess the influence of acute and extended exposure to cigarette smoke on the clearance of inhaled bacteria from the respiratory tract (see Addendum I, Supporting Data, pages 19 to 33.

B. Conditions of In Vitro Smoke-Exposure: A commercial brand of non-filtered cigarette is attached to a 30 ml syringe by a rubber tube and smoke is produced by withdrawing the barrel of the syringe at a rate of approximately 18 ml per second. Six successive "puffs" of cigarette smoke are introduced into the syringe and emptied by removing a rubber tube containing the lighted cigarette. The smoke from the seventh puff is introduced into the tissue culture flasks with a sterile hypodermic needle. The protocol of each study included a control flask (bacteria only) and macrophage cultures not exposed to cigarette smoke and control and corresponding macrophage preparations exposed to cigarette smoke. Under these conditions, macrophage cultures are exposed for 1.5 hours to whole cigarette smoke.

C. Conditions of In Vitro Bacterial Challenge: Alveolar macrophages harvested from rabbits are placed in tissue culture flasks containing a tissue culture medium (Hanks' solution) and normal serum, immune serum, normal bronchopulmonary lavage, immune bronchopulmonary in accordance with the protocol of each study. Immediately after inoculation with fixed numbers of Staphylococcus aureus (FDA 209P, phage type 42D) and at 15 min intervals over a 90 min period after bacterial challenge, the macrophage cultures are processed to measure overall antibacterial activity, phagocytosis and bacterial destruction as outlined on pages 24-25. To determine if the effects of in vivo smoke-exposure

*Progressive Engineering Co., Richmond, Va.

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on macrophage function are reversible, overall antibacterial activity, phagocytosis and bacterial destruction will be measured immediately following exposure to cigarette smoke for 1, 5, 10 and 15 days and 1, 5 and 10 days after the termination of experimental smoke inhalation.

D. Harvesting of Alveolar Macrophages: Alveolar macrophages are harvested from the lungs of albino rabbits weighing 1.0 to 2.0 kilograms by the general method of Myrvik et al(13). The animals are killed by injecting air in the marginal ear vein. This method of sacrificing animals is used to avoid any depressant effects that anesthetics may have on alveolar macrophage activity. The trachea is exposed and canulated with a sterile polyethylene tube, and 17 ml of sterile Hanks' solution is introduced in the intact lung. It is allowed to remain in the lung for 6 min, and then is recovered by aspiration. The lavage fluid is centrifuged at 2000 rpm for 10 min. The resultant supernatant fluid is separated from the cell pellet by decanting thus dividing the aspirated material into an alveolar macrophage fraction and an acellular fluid fraction containing the immunologic contents of the lung retrievable by bronchopulmonary lavage. The acellular fluid fraction is stored at - 70°C and later processed as described below (see E). The numbers of macrophages present in the macrophage fraction are enumerated in a bright line hemocytometer and differential counts are made on Wright stained smears. By this method 95% of the macrophages harvested are viable as determined by the Eosin Y dye exclusion test (14).

E. Processing of Bronchopulmonary Lavage to Characterize Immunologic Components Extractable from the Lung: The acellular fraction of lung harvests (see above D) will be concentrated 5, 10 and 50 fold by ultrafiltration in Diaflow Membranes (Amicon Corp.). To date this method of processing the acellular fraction of the lung has resulted in the recovery of bronchopulmonary lavage material capable of promoting the phagocytic activity of alveolar macrophages in the absence of exogenous serum.

F. Immunization Schedule:

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(1) Parenteral Immunization: Antiserum specific to S. aureus will be produced in rabbits as presented by Oeding (15). A suspension of 2 to 4 x 10^9 staphylococci/ml is centrifuged at 6,000 rpm for 20 min and the resultant supernatant is discarded. The pellet containing staphylococci is resuspended in 4% formalized saline and stored at 4°C for 24 hrs. At this time, the formalin-treated bacteria are streaked out on blood agar to test for the presence of viable staphylococci. If bacterial growth is detected, the formalin suspension of bacteria is stored at 4°C for 24 hr intervals until total bacterial death is achieved. The suspension is then washed and centrifuged 3 times and resuspended in saline. The rabbits are treated with the formalin killed bacteria according to the following schedule. Rabbits are injected intravenously (iv)

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on 3 successive days with 0.1 ml, 0.2 ml and 0.4 ml of the formalized S. aureus. After 5 days the rabbits are now injected iv with 0.4 ml, 0.6 ml and 0.8 ml of formalin-killed staphylococci on 3 successive days. Five days later the rabbits receives iv injections with 0.8 ml, 1.0 ml and 1.0 ml of formalin-treated S. aureus on three successive days. Finally, five days after the termination of the last set of intravenous inoculations with formalin-killed bacteria, the animals are bled and serum reactivity is tested by double immunodiffusion against S. aureus. Upon confirmation of the achievement of a high titer, all the animals will be exsanguinated by cardiac puncture. In addition, at this time, lungs of the sacrificed rabbits will be washed out by bronchopulmonary lavage, concentrated and tested for activity against S. aureus as outlined above

(2) Intranasal Immunization: Since it has been shown that the intranasal or aerogenic route of immunization favors the production of SIgA agglutinative antibody on bronchial secretions in response to a given antigen (10) this method will be used for the purpose of studying the influence of SIgA on the phagocytic capacity of alveolar macrophages. Rabbits will be immunized with heat-killed S. aureus (10^9 organisms/ml) by intranasal inoculation utilizing the procedure and immunization schedule of Reynolds et al (15). In short, 2 ml of S. aureus suspension will be instilled intranasally with an eye dropper 3 times per week for 2 weeks or until agglutinating antibodies are detected in bronchial lavage material.

G. Quantitation of C'3, C'5, IgG and SIgA: Quantitation of the immunoglobulins and complement proteins in serum will be performed by the method of electroimmunodiffusion as outlined by Merrill et al (17). The antirabbit IgG, C'3 and C'5 sera and the appropriate antigen standards needed for the procedure will be purchased from Microbiological Associates. The same methodology will be used to quantitate C'3, C'5 and IgG in bronchopulmonary lavage material. In addition anti SIgA and SIgA standards previously prepared in this laboratory by the method of Cebra and Robins (18) will be used to quantitate the amount of SIgA extractable from the lungs by bronchopulmonary lavage.

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With regards to the electroimmunodiffusion method proper, glass slides (1" x 3") are coated with 0.3% Ionagar containing 0.05% glycerol. The slides are then coated with 2 ml of Agarose in a vernol buffer (pH 8.6, 0.05 M) containing multiple dilutions of antiserum against the specific antigen under study. After the slides solidify, 3 circular wells are cut into the agar along the short axis of each slide and filled with 4 ul of the test serum or bronchial fluid. The slides are now subjected to electrophoresis for 90 min at 150 volts. After electrophoresis the slides are washed in saline and distilled water for 1 hr and finally dried at 37°C. The dried slides

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are stained with 0.1% Amido Schwartz in 1.0% acetic acid. The length of the resultant precipitin lines formed by the migration of the antigens present in serum or bronchial lavage is measured and plotted against the precipitin lines formed by standard antigens of known concentration. A new standard curve will be constructed for each humoral component studied.

H. Selective Depletion/Absorption of C'3, C'5, IgG and SIgA: Depletion of C'3 will be attained by reacting serum or bronchial lavage material with Zymosan (1.35 mg/ml) at 37°C for 1 hr (18). The test serum or bronchopulmonary lavage material is centrifuged and the supernatant free of C'3 is recovered.

The absorption of C'5, IgG and SIgA will be performed by modifications of a standard immunoadsorption technique described in detail in published reports (20,21). Sepharose 4B is swelled, equilibrated in an appropriate buffer and coupled with either anti C'5, IgG or SIgA sera at 4°C on a rotary shaker overnight. The antibody-gel complex is placed in a beaker containing serum or bronchopulmonary lavage material and mixed gently for 2 hrs at room temperature. The suspension is centrifuged at 2,500 rpm for 20 min at 4°C to pellet the Sepharose beads containing the antigen-antibody complex. The supernatant free of C'5, IgG or SIgA is decanted and filter-sterilized.

The completeness of depletion or absorption of C'3, C'5, IgG and SIgA achieved by the methods presented above will be monitored by double immunodiffusion against appropriate antisera. Failure of serum and bronchopulmonary lavage material to react under conditions of Ouchterlony analysis will be used as an index of the completeness of depletion or absorption. Since the reaction between IgG and anti IgG is complement dependent, serum and bronchopulmonary lavage levels of complement will also be measured.

I. Equivalence Point Titrations: Equivalence points will be determined by the method described by Campbell et al (22). Serial dilutions are made of antisera to C'3, C'5, IgG and SIgA and a fixed amount of serum or bronchopulmonary lavage material will be added to each antisera dilution. The highest antisera dilution showing the greatest amount of precipitation is considered the zone of equivalence. The time of the reaction is also noted.

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16. Reynolds, H. Y., Thompson, R. E. and Devlin, H. B.: Development of cellular and humoral immunity in the respiratory tract of rabbits to pseudomonas lipopolysaccharide. J. Clin. Invest. 53: 1351, 1974.

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18. Cebra, J. and Robbins, J.: Gamma A immunoglobulin from rabbit colostrum. J. Immunol. 92: 12, 1966.

19. Weir, D.M.: Handbook of Experimental Immunochemistry. Blackwell Scientific Publications, Oxford, 1973.

20. Frommel, D., Dupuy, J.M. and Litman, G.W., et al.: Use of immunoadsorption technique in the preparation of chemical agammaglobulinemia. J. Immunol. 105: 1292, 1970.

21. CNBr - Activated Sepharose 4B for Immobilization of Biopolymers. Pharmacia Fine Chemicals, Inc., Piscataway, N. J., 1973.

22. Campbell, D. H., Garvey, J. S., Cremer, N. E. and Susdorf, D. H. Methods in Immunology. 2nd ed. W. A. Benjamin, Inc., New York, 1970.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The Microbiology Research Laboratories are located as a complex in the Triboro Hospital at Queens Hospital Center, Jamaica, New York. They include: (a) an aerosol exposure laboratory, (b) a smoke-exposure laboratory, (c) individual laboratories for macrophage studies and microbiology, (d) 2 rooms for storage space and refrigerators. Animal quarters are provided in another area of the hospital. The entire laboratory area occupies approximately 700 square feet.

The equipment in this area includes a complete bacterial aerosol generating and exposure system with mixing chambers and decontaminating units, and an Anderson apparatus for measuring particle size of bacterial aerosols: A cigarette smoke generating apparatus and exposure chamber and a sequential sampler and gas liquid chromatography unit for determining concentration of the particulate and gas phase of cigarette smoke. Other major instrumentation present include the following: (a) standard microscopes, (b) 1 infusion pump, (c) a centrifuge, (d) 2 large refrigerators, (e) sonic dismembranator, (f) 2 water baths and shaker, (g) 1 freezer, (h) 2 incubators and 1 environmental chamber, (i) 4 vacuum pumps, (j) pH meter, (k) Beckman DU2 recording spectrophotometer, (l) spectronic 20 spectrophotometer, (m) Gilson respirometer, (n) a lyophilizer unit, (o) immuno and disc electrophoresis apparatus, (p) large autoclave, (σ) analytical balance, (r) flash evaporator and (s) Isco automatic fraction collector for liquid chromatography. There is also equipment for qualitative and quantitative bacteriology studies, tissue homogenation, administration of gas mixtures and animal surgery. A Revco deep freezer (-75°C) is also available. High performance scintillation counters capable of isotope work are present in the hospital and available for research use. In addition, a fully equipped laboratory capable of performing histological and electron microscopy studies are available to the Division of Microbiology.

11. Additional facilities required:

None.

12. Biographical sketches of investigator(s) and other professional personnel (append):

See pages 6 - 13.

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

See pages 14 - 15 and Addendum II.

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14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Joseph J. Guarneri, Ph.D. Principal Investigator	20 hrs/60 hrs	REDAC
Boris A. Shidlovsky, Ph.D.	7 hrs	REDACTED
Technical		
Research Associate (to be appointed)	35 hrs/100%	$15,600
	Fringe Benefits	2,808
	Sub-Total for A	18,408

B. Consumable supplies (by major categories)

See page 4A for detailed list

Sub-Total for B	4,850

C. Other expenses (itemize)

Journals and Books	200
Travel	800
Sub-Total for C	1,000
Running Total of A + B + C	24,258

D. Permanent equipment (itemize)

None

Sub-Total for D	-
E	3,369
Total request	27,897

E. Indirect costs (15% of A+B+C)

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	19,881	4,800	1,000	-	4,002	29,534
Year 3	21,471	4,800	1,000	-	4,090	31,362

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B. Consumable Supplies

1.	Cigarettes for smoke studies	$1,000
2.	Mice	100
3.	Rabbits	2,000
4.	Immunodiffusion Plates, Antisera, Radial Immunodiffusion kit and templates, Antigen standards	600
5.	Tissue culture glassware, tissue culture media and bacteriology media, and nebulizers	550
6.	Petri dishes and plastic disposable pipets and bacteriologic filters	600
		$4,850

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16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophages and Mucociliary Function.	The Council for Tobacco Research, U.S.A. Grant Nos. 547C, 547 CR-1 and 547 CR-2	$65,152	7/1/71 to 6/30/75
Important Determinants of Pulmonary Resistance to Infection, Alcoholic Intoxications.	Long Island Jewish-Hillside Medical Center Grant No. 274	33,600	5/3/74 to 12/31/75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
None	Renewal of LIJ/Hillside Medical Center Grant No. 274	25,000	1/1/76 - 12/30/76

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name: Joseph J. Guarneri, Ph.D.

Signature: Joseph J. Guarneri Date: 7/16/75

Telephone: REDACTED (Area Code / Number / Extension)

Checks payable to

Long Island Jewish-Hillside Medical Center

Mailing address for checks

Mrs. Eva Meyer, Grant Manager
Long Island Jewish-Hillside Medical Center
New Hyde Park, New York 11040

Responsible officer of institution

Typed Name: James E. Mulvihill, D.M.D.

Title: Vice President Research and Education

Signature: James E. Mulvihill D.M.D. Date: 7-16-75

Telephone: REDACTED 2111 (Area Code / Number / Extension)

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12 Biographical Sketch Joseph J. Guarneri, Ph.D. Principal Investigator

NAME: Joseph J. Guarneri, Ph.D.

ADDRESS: HOME: REDACTED

OFFICE: Long Island Jewish-Hillside Medical Center/Queens Hospital Center
82-68 164th St., Jamaica, New York 11432

TELEPHONE: HOME: REDACTED
OFFICE: 212-990-2335

BIRTHDATE AND PLACE: REDACTED

CITIZENSHIP: REDACTED

MARITAL STATUS: REDACTED

SOCIAL SECURITY #: REDACTED

EDUCATION: REDACTED New York University; B.A. (Biology), 6/49
- Saint John's University; M.S. (Microbiology), 6/63
REDACTED - Saint John's University; Ph.D. (Microbiology), 6/66

INTERNSHIP RESIDENCY: Not applicable.

MILITARY SERVICE: 1/49 - 3/46 - Sergeant, U.S. Army; Medical Corp., Camp Lee, Virginia

BOARD STATUS: American Society for Microbiology; Certification as Specialist in Public Health and Medical Laboratory Microbiology.
American Academy of Microbiology; Fellowship (Pending).

TYPE OF PRACTICE: Not applicable. 1003545981

LICENSURE STATUS: Certificate of Qualification for Director of a Clinical Microbiology Laboratory, City of New York, Department of Health.

ACADEMIC POSITIONS:

9/61 - 6/66 - Research Associate, Division of Respiratory Diseases, New Jersey College of Medicine and Dentistry.

6/66 - 6/68 - Instructor in Medicine, Department of Medicine, New Jersey College of Medicine and Dentistry, Jersey City, New Jersey.

7/68 - 1/72 Director, Pulmonary Aerobiology Research Laboratory, Division of Infectious Diseases, Department of Medicine, Saint Vincent Hospital Worcester, Mass.

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ACADEMIC POSITIONS:

1/72	Attending Microbiologist, Long Island Jewish-Hillside Medical Center/Queens Hospital Center.
6/73	Associate Clinical Professor Pathology, SUNY at Stony Brook.
6/73	Coordinator Allied Health Sciences, Queens Hospital Center, Jamaica, New York.
9/73	Associate in Microbiology, St. John's University, Jamaica, New York.

MEMBERSHIP IN PROFESSIONAL SOCIETIES:

REDACTED

REDACTED

REDACTED

REDACTED

HONORS AND AWARDS:

Sigma XI, Saint John's University, 1963

3/68 to 5/69 - Public Health Service. H.I.H. Award #A.I. 08963-01. Title: The Mechanism of Pulmonary Resistance to Infection. Principal Investigators: G.A. Laurenzi, M.D. Associate Director: J.J. Guarneri, Ph.D. - Amount $67,769.

7/68 to 6/69 - The Council for Tobacco Research-U.S.A. Grant Award #547. Title: The Effect of Cigarette Smoke on the Nature and Function of Alveolar Macrophages. Principal Investigator: G.A. Laurenzi, M.D. Co-Investigator: J. J. Guarneri, Ph.D. - Amount $36,135.

9/68 to 6/71 - Saint Vincent Hospital Research Foundation. Title: The Role of the Alveolar Macrophage in Pulmonary Defense Against Inhaled Bacteria. Principal Investigator: J. J. Guarneri, Ph.D. Amount: $ 18,667.

6/69 to 5/72 - Public Health Service. H.I.A. Award #AI 08963-02. Title: The Mechanism of Pulmonary Defense Against Infection. Principal Investigator: G.A. Laurenzi, M.D. Associate Director: J.J. Guarneri, Ph.D. - Amount: $128,137.

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HONORS AND AWARDS:

7/69 - 6/71 - The Council for Tobacco Research - U.S.A. Grant Award #547BR1. Title: The Effect of Cigarette Smoke on the Immunological and Metabolic Function of Alveolar Macrophages. Principal Investigator: J.J. Guarneri Ph.D. - Amount: $29,380.

7/71 - 6/72 - Saint Vincent Hospital Research Foundation. Title: The Influence of Bacterial Species on the Antibacterial Activity of Alveolar Macrophages. Principal Investigator: J. J. Guarneri, Ph.D. Amount $ 4,000.

7/71 - 6/75 - The Council for Tobacco Research - U.S.A. Grant Award # 547C. Title: The Influence of Extended Exposure to Cigarette Smoke on Pulmonary Resistance to Infection as Related to Alveolar Macrophage and Mucociliary Function. Principal Investigator: J.J. Guarneri, Ph.D. - Amount: $63,000.

5/74 - 6/75 - Long Island Jewish-Hillside Medical Center #274. Title: Important Determinants of Pulmonary Resistance to Infection, Alcoholic Intoxication. Principal Investigator: J. J. Guarneri, Ph.D. - Amount: $22,413.

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Source: https://www.industrydocuments.ucsf.edu/docs/gpdm0000

PUBLICATIONS: (Papers published or in press).

1. Laurenzi, G.A., Guarneri, J.J., Endriga, R.B. and Carey, J.P.: Clearance of Bacteria by the Lower Respiratory Tract. Science: 142: 1572-1573, 1963.

2. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B. "Important Determinants in Pulmonary Resistance to Bacterial Infections." In the Pathogenisis of Chronic Obstructive Broncho-Pulmonary Disease. In Mitchell R.S.: Progress in Research in Emphysema and Chronic Bronchitis, New York, S. Karger, 1965, p. 48-59.

3. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Important Determinants in Pulmonary Resistance to Bacterial Infection. Medicinia Thoracalis 22: 48-59, 1965.

4. Laurenzi, G.A., and Guarneri, J.J.: A Study of the Mechanisms of Pulmonary Resistance to Infection: The Relationship of Bacterial Clearance to Ciliary and Alveolar Macrophage Function. Symposium on Structure, Function, and Measurement of Respiratory Cilia. Am. Rev. of Respiratory Dis. 93: 134-141, 1966.

5. Laurenzi, G.A., Yin, S., Collins, R., Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Current Research in Chronic Obstructive Lung Disease. U.S. Public Health Service Publication No. 1787: 27-40, 1967.

6. Laurenzi, G.A., Yin, S., and Guarneri, J.J.: The Adverse Effect of Oxygen on Tracheal Mucus Flow. New England J. of Med. 279: 333-339, 1968.

7. Guarneri, J.J. and Laurenzi, G.A.: The Effect of Alcohol on the Mobilization of Alveolar Macrophages. J. of Lab. and Clinical Med. 72: 40-51, 1968.

8. Combs, T.J., Guarneri, J.J., and Pisano, M.A. The Effect of Sodium Chloride on the Lipid Contents and Fatty Acid Composition of Candida Albicans. Mycologia LX: 1232-1239, 1968.

9. Guarneri, J.J.: Clearance of Inhaled Bacteria from the Murine Respiratory Tract. In Developments In Industrial Microbiology. American Institute of Biological Sciences, Washington, D.C., Volume 16, 1974

10. Khan, F., Guarneri, J.J. and Sierra, M.F.: Primary Pulmonary Sporotrichosis Complicated by Perirectal. Am. Rev. Respiratory Dis., 1975

11. Guarneri, J.J.: Influence of Acute Exposure to Cigarette Smoke on the Alveolar Macrophage System. Submitted to J. of Lab. and Clin. Med.

ABSTRACTS: (abstracts published and presented or read by title) **1003545984**

1a. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Bacterial Clearance from the Lung of Mice, Fed. Proc.: 22: 255, 1963.

2a. Laurenzi, G.A., Endriga, R.B., Guarneri, J.J. and Carey, J.P.: Important Determinants in Resistance to Pulmonary Infection. J. Clinical Invest. 949: 42, 1963.

3. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Important Determinants in Pulmonary Resistance to Infection: Proceeding of Seventh Aspen Conference on Bronchitis and Pulmonary Emphysema. p. I-4, 1964.

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4a. Laurenzi, G.A., Collins, B.J., Yin S. and Guarneri, J.J.: The Adverse Effects of High Oxygen Breathing and Hypoxia. J. Clin. Invest. 45: 1035, 1966.

5a. Laurenzi, G.A., Yin, S., Collins, B.J. and Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Proceeding of the Tenth Aspen Conference. p. 100, 1966.

6a. Laurenzi, G.A., Collins, B.J., Yin, S. and Guarneri, J.J.: Adverse Effect of High Oxygen Breathing on Tracheobronchial Mucus Flow. Amer. Rev. Resp. Dis. Vol. 96: 152, 1967.

7a. Guarneri, J.J., Combs, T.J. and Pisano, M.A.: Lipid Components of Candida Stellatoide Bacterial Proc. p. 84, 1967.

8a. Guarneri, J.J.: Lipid Composition of Candida Stellatoides. Dissertation Abstracts 27: 3614-B, 1967.

9a. Guarneri, J.J. and Laurenzi, G.A.: The Mobilization of Alveolar Macrophages as a Pulmonary Defense Mechanism Against Inhaled Bacteria. Bacterial Proc. p. 100, 1968.

10a. Laurenzi, G.A., Yin, S., Collins, B.J. and Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Public Health Service Publication No. 1787, p. 27, 1967.

11a. Combs, T.J., Guarneri, J.J. and Pisano, M.A.: Effect of Growth Conditions on the Fatty Acid Composition of Candida Albicans. The Third Symposium on Yeasts, Delft-Hague, The Netherlands, June 2 - 7, 1969.

12a. Guarneri, J.J. and Laurenzi, G.A.: The Effect of Cigarette Smoke on Alveolar Macrophage Numbers. The 148th Annual Meeting of the New York City Branch of the American Society for Microbiology, New York, N.Y., Feb. 25, 1971.

13a. Guarneri, J.J. and Laurenzi, G.A.: Influence of Cigarette Smoke on Pulmonary Defense Against Inhaled Bacteria. Alveolar Macrophage Numbers and Viability, The 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., May 26, 1972.

14a. Guarneri, J.J. and Sierra, M.F.: Antibacterial Activity of Alveolar Macrophages Against Staphylococcus aureus. The 197th Annual Meeting of The American Society for Microbiology, New York City Branch, Wagner College, Staten Island, New York.

15a. Guarneri, J.J.: Influence of In vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. The 197th Annual Meeting of The American Society for Microbiology, New York City Branch, Wagner College, Staten Island, New York, April 15, 1974.

16a. Guarneri, J.J.: Influence of Extended Exposure to Cigarette Smoke on Pulmonary Defense against Inhaled Bacteria. 74th Annual Meeting of The American Society for Microbiology, Chicago, Ill., May 12- 17, 1974.

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17a. Guarneri, J.J.: Clearance of Inhaled Bacteria from Murine Respiratory Tract. 25th Annual Meeting of the Society for Industrial Microbiology, Memphis, Tenn., Aug. 11 - 16, 1974. (Abstract)

18a. Guarneri, J.J.: Influence of Acute Exposure to Cigarette Smoke on Pulmonary Defense Mechanisms. 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif. Sept. 11 - 13, 1974. (Abstract).

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19a. Guarneri, J.J.: Influence of In Vivo Exposure to Whole Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. 1975 Annual Meeting of American Society for Microbiology.

20a. Guarneri, J.J., Goldstein, J. and Shidlovsky, B.A.: Effect of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. 1975 Annual Meeting of American Society for Microbiology.

21a. Guarneri, J.J., Laurenzi, G.A., and Sierra, M.F.: Influence of Trichlorofluoromethane and Dichlorodifluoromethane on the Clearance of Bacteria from the Lungs of Mice. 26th Annual Meeting of the Society for Industrial Microbiology, Kingston, R.I., August 17th - 22nd, 1975.

PRESENTATIONS: (papers given by invitation and thesis)

1p. Guarneri, J.J.: The Inhibition of Bacteria by Aconitic Acid. Master's Thesis, St. John's University, June 9, 1963.

2p. Laurenzi, G.A., Guarneri, J.J. and Endriga, R.B.: Important Determinants in Pulmonary Resistance to Infection: Processing of Seventh Aspen Conference on Bronchitis and Pulmonary Emphysema, p. 1 - 4, 1964.

3p. Laurenzi, G.A., Yin, S., Collins, B.J. and Guarneri, J.J.: Mucus Flow in the Mammalian Trachea. Proceeding of the Tenth Aspen Conference, p. 100, 1966.

4p. Guarneri, J.J.: Lipid Composition of Candida Stellatoidea. Ph.D. Thesis, St. John's University, June 12, 1966.

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R: REDACTED MATERIAL

Biographical Sketch - Boris A. Shidlovsky, Ph.D.

NAME: Boris A. Shidlovsky, Ph.D.

REDACTED

ADDRESS: Home: -
Office: Long Island Jewish-Hillside Medical Center/
Queens Hospital Center
82-68 164 Street, Jamaica, New York 11432

TELEPHONE: Home: REDACTED
Office: 212-990-2337

DATE OF BIRTH:

MARITAL STATUS: REDACTED

EDUCATION:

R - New York University B.A.
- St. John's University M.S.
- St. John's University Ph.D.

MILITARY SERVICE: 1942 - 1946 U.S. Army Medical Corps. senior non-commissioned officer.

LICENSURE STATUS: Certificate of Qualification for Director of a Clinical Microbiology Laboratory, City of New York, Department of Health.

PROFESSIONAL EXPERIENCE:

1951-1961	Div. of Surg. Res. Lab. Harlem Hospital, New York City, Bacteriologist-in-charge.
1961-1962	Microbiology Department, New York University Dental School, New York, Research Associate.
1962-1963	Misericordia Hospital, New York, Chief Bacteriologist
1963-1966	Morrisania Hospital (Montefiore-Morrisania Hospital Affil.), Chief Bacteriologist
1966-1969	Quinton Research Labs/Merck & Co., Inc. (Senior Research Microbiologist)
1969-1974	Associate Professor at Monmouth College, West Long Branch, New Jersey
1974	Assistant Attending Microbiologist, Long Island Jewish-Hillside Medical Center/ Queens Hospital Center.

MEMBERSHIPS IN PROFESSIONAL SOCIETIES:

REDACTED

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MEMBERSHIPS IN PROFESSIONAL SOCIETIES:

New York City Branch-Am. Soc. Microbiol.
Society of General Microbiology
Society of Industrial Microbiology
SIGMA Xi Club of St. John's University
Theobald Smith Society

RESEARCH INTERESTS: Antimicrobial agents and host defense mechanisms

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13. Publications Pertinent to Material Covered in Grant Proposal*

1. Guarneri, J.J. and Laurenzi, G.A. The Effect of Cigarette Smoke on Alveolar Macrophage Numbers. The 148th Annual Meeting of the New York City Branch of the American Society for Microbiology, New York City, Feb. 25, 1971.

2. Guarneri, J.J. and Laurenzi, G.A. Influence of Cigarette Smoke on Pulmonary Defense Against Inhaled Bacteria: Alveolar Macrophage Numbers and Viability. The 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., May 26, 1972. (Abstract No. M 170).

3. Guarneri, J.J. and Sierra, M.F. Antibacterial Activity of Alveolar Macrophages Against Staphylococcus aureus. The 1974 Annual Meeting of the American Society for Microbiology, New York City Branch, New York, April 15, 1974.

4. Guarneri, J.J. Influence of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. The 1974 Annual Meeting of the American Society for Microbiology, New York City Branch, New York, April 15, 1974.

5. Guarneri, J.J. Influence of Extended Exposure to Cigarette Smoke on Pulmonary Defense against Inhaled Bacteria. 74th Annual Meeting of the American Society for Microbiology, Chicago, Ill. May 12 - 17, 1974. (abstract No. M 355).

6. Guarneri, J.J. Clearance of Inhaled Bacteria from the Murine Respiratory Tract. The 25th Annual Meeting of the Society for Industrial Microbiology, Memphis, Tenn., Aug. 11 - 16, 1974.

7. Guarneri, J.J. Influence of Acute Exposure to Cigarette Smoke on Pulmonary Defense Mechanisms. The 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., Sept. 11-13, 1974. (Abstract No. 181).

8. Guarneri, J. J. Clearance of Inhaled Bacteria from the Murine Respiratory Tract. In: Developments In Industrial Microbiology. American Institute of Biological Sciences, Washington, D.C., Volume 16, 1975.

9. Guarneri, J.J. Influence of Acute Exposure to Cigarette Smoke on the Clearance of Bacteria by the Murine Respiratory Tract. Presented to CTR for Review Prior to Submission to Am. Rev. Resp. Dis.

10. Guarneri, J.J. and Goldstein, J. A study of the In Vitro Interaction Between Alveolar Macrophages and Staphylococcus aureus. The 1975 Annual Meeting of the American Society for Microbiology, New York City Branch, New York. Jan. 14, 1975.

11. Guarneri, J.J. Influence of In Vivo Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. Abstracts of the Annual Meeting of the American Society for Microbiology, 1975 (Abstract No. B 43).

12. Guarneri, J.J., Goldstein, J. and Shidlovsky, B.: Effect of In Vitro Exposure to Cigarette Smoke on the Antibacterial Properties of Alveolar Macrophages. Abstracts of the Annual Meeting of the American Society for Microbiology, 1975 (Abstract No. B 44).

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13. Publications Pertinent to Material Covered in Grant Proposal*

13. Guarneri, J.J. Influence of Acute Exposure to Cigarette Smoke on the Clearance of Bacteria by the Murine Respiratory Tract. Presented to the Council for Tobacco Research Prior to Submission to Am. Rev. Resp. Dis.

14. Guarneri, J.J. Influence of Extended Exposure to Cigarette Smoke on the Clearance of Bacteria by the Murine Respiratory Tract. (In Preparation).

* See Curriculum Vitae page 9 for a complete list of publication.
See Addendum II for copies of publications pertinent to material covered in grant proposal.

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